# The Effect of Temperature on D-lactate Production during Male Courtship in the Brush-legged Wolf Spider *Schizocosa ocreata*

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Wolf spiders (Lycosidae), like all spiders, are ectothermic, meaning that their body temperature is similar to the temperature of the surrounding environment. This puts added constraints on their ability to perform both aerobically and anaerobically. Thus, temperature directly affects behavioral vigor (performance) which is tied to reproductive success in wolf spiders. It stands to reason that individuals with greater performance capacity (higher respiration limits), regardless of temperature, should achieve greater mating success. In this experiment we will be analyzing the production of D-lactate (a byproduct of anaerobic metabolism in wolf spiders) in tissues of the brush-legged wolf spider, *Schizocosa ocreata*. Males of this species actively seek and court females in the leaf litter habitat of eastern deciduous forests during the spring breeding season. Males are regularly exposed to thermal variation (sun/shade) as they move through the litter and it has been suggested that they are behaviorally maintaining body temperature above ambient, which would increase behavioral vigor and potentially increase mating opportunities. The objective will be to establish D-lactate production levels (minimum and maximum) for males at predetermined substrate temperatures that represent the seasonally appropriate thermal minimum, mean, and maximum in the natural habitat. The resulting estimates of optimum anaerobic performance temperature will confirm that there is variance among males in anaerobic performance, and further, will allow us to better predict what thermal sites males should pick to optimize courtship. Ultimately, we will be able to explore whether the anaerobic capacity of preferred males is higher than less preferred males, in this as of yet unexplored aspect of sexual selection in this well-studied animal model.

## INTRODUCTION AND REVIEW OF LITERATURE

Physiological ecology has become an important topic of investigation in recent years, especially the link between the physiology and behavioral ecology. An animal's behavioral performance, ability to evade predators, compete for food, or even reproduce, are all dependent on its underlying physiology. Behavioral performance (vigor) is closely tied to an organism's likelihood of reproduction; which may be temperature sensitive (Li & Jackson 1996; Jaio et al. 2009), especially in small ectotherms where behavior is largely dependent on ambient and substrate temperatures (Costa & Sotelo 1984; Schmalhofer 1999; Jaio et al. 2009; Schmalhofer 2011). Behavioral performance studies often demonstrate relationships between performance and reproductive success, but frequently lack considerations of the complexities of biochemical mechanisms by which performance may be constrained (Ellington 1983; Clarke & Fraser 2004). Thus, physiological ecology studies require a strong understanding of individual activity variation within a population, and ultimately individual physiological constraints within a population, in order to accurately extrapolate their role species wide.

The arachnids are one of the largest groups in the animal kingdom with currently around 42,000 described species (Merrett et al. 2011). They are found in nearly every habitat, from old growth forest to deserts, and play a strong role in most food webs as a top predator (Foelix 2010). However despite their great numbers and ecological importance, the amount of data available on arachnid physiology is limited (Barth 2002). Wolf spiders (Lycosidae) are one of the best studied spider groups and are fast becoming a favorite model of behavioral ecology research (Uetz & Roberts 2002; Hebets & Papaj 2005; Foelix 2010). This is especially true of

members of the genus *Schizocosa* which have been widely used in studies of multimodal communication, signal evolution, and mate choice (Uetz 2000; Uetz & Roberts 2002; Hebets & Papaj 2005). *Schizocosa ocreata* males actively seek and court females in the leaf litter habitat of eastern deciduous forests during the spring breeding season (Cady 1984). Males are regularly exposed to thermal variation (sun/shade) as they move through the litter and it has been suggested that by moving in and out of sun flecks, they are behaviorally maintaining body temperature above ambient, which would increase behavioral vigor and potentially increase mating opportunities (Clark, Roberts, & Uetz unpubl). Behavioral parameters such as courtship latency and duration, in the wolf spider *Pardosa astringera* have been shown to be highly dependent on environmental temperature (Jaio et al. 2009). Thus, substrate temperature is likely to directly affect behavioral vigor (performance), which is tied to reproductive success in wolf spiders. It stands to reason that, regardless of temperature, individuals with greater performance capacity (higher aerobic and anaerobic respiration limits) and shorter recovery times should be able to tolerate performing at a level closer to the species maximum, resulting in greater mating success. Like all spiders, the *Schizocosa* are ectothermic, meaning that their body temperature is similar to the temperature of the surrounding environment (Foelix 2010). Thus, colder temperatures should mean correspondingly reduced behavioral activity, as has been found in other wolf spiders (Costa & Sotelo 1984; Davis 1989; Ackers, Zajd, & Roberts unpubl).

Given that spiders are relatively small-bodied ectotherms, temperature may be the most critical environmental factor regulating key behaviors, such as courtship and copulation, and there is likely a range of temperatures over which spiders perform optimally (Davis 1989; Li et

al. 1996; Jaio et al. 2009; Ackers, Zajd, & Roberts unpubl). Gillooly et al. 2001 discussed the effects of size and temperature on resting metabolic rate (RMR). Researchers suggest that both temperature and body mass are the major interacting factors affecting RMR. Ectotherms (fish, amphibians, and other invertebrates) and endotherms (birds, and mammals) both show a negative relationship between mass-normalized RMR and temperature. Conversely, the relationship between temperature normalized (20°C) RMR and mass was found to have a positive relationship. This implies that smaller organisms, such as spiders, may rely more heavily on temperature to help regulate their basal or resting metabolic rate. Knowledge of how metabolic rate is affected by body mass and temperature helps to understand how animals regulate the utilization of stored energy towards various activities necessary for survival of the organism. The roles of body size and ambient temperature in influencing ectotherm activity have previously been established (Schmalhofer 1999; Gillooly et al. 2001; Meehan 2006). There has been increasing interest in the role of temperature in affecting small ectotherm behavior and performance, such as wolf spiders (Li & Jackson 1996; Jaio et al. 2009; Roberts et al. unpubl). Furthermore, temperature in ectotherms was found to show significant negative correlation with resting metabolic rate (Clarke & Fraser 2004; Gilooly et al. 2001). That is, as temperature increased, a decrease in resting respiration rates occurred. Although this relationship has been investigated less extensively in terrestrial ectotherms (Addo-Bediako et al. 2002), temperature has been shown to have a weak but significant negative correlation with metabolic rate in small ectothermic insects (Clarke & Fraser 2004). These similar interspecies findings suggest a level of cold adaptation present in small ectotherms, and likely translate to other ectotherms, such as arachnids (Anderson 1996).

There is a great deal of diversity in respiratory physiology within spiders; some have book lungs and trachea, some have trachea only, and still others have book lungs with non-functional trachea (Levi 1967; Anderson & Prestwich 1982; Schmitz 2004; Schmitz 2005). Wolf spiders rely primarily on book lung function and are poorly tracheated compared to other species of spiders (Schmitz 2004). The lack of well-developed trachea leads to less surface area for gas exchange and increased reliance on anaerobic respiration during high activity; resulting in an inverse correlation between anaerobic capacity and the respiratory organ surface area (Linzen & Gallowitz 1975; Prestwich & Ing 1982, Prestwich 1983a, b; Schmitz 2004). The diffusion of oxygen across book lung leaflets depends upon binding and affinity to hemocyanin (oxygen transport protein in spiders) (Foelix 2010). It has been shown that hemocyanin in spiders is only ~50% saturated with oxygen prior to activity, and upon activity oxygen is rapidly depleted (Angersbach 1978; Paul et al. 1994 a, b). This suggests that wolf spiders quickly deplete their oxygen stores and must rely on anaerobic metabolism to generate adequate energy for activity. Due to wolf spider physiology and "sit-and-wait" style of prey capture, they do not regularly perform highly aerobic activities (Levi 1967; Prestwich 1977; Prestwich 1983a; Paul et al. 1989). Wolf spiders often endure short, high intensity bursts of activity in the course of prey capture or escape from predators, in order to compensate for their low aerobic abilities, have reasonably well developed anaerobic capacities (Linzen & Gallowitz 1975; Prestwich & Ing 1982, Prestwich 1983a, b; Schmitz 2004; Schmitz 2005). Because behavioral vigor (performance) is tied to reproductive success in arachnids; it stands to reason that individuals with greater performance capacity (higher aerobic and anaerobic respiration limits) should achieve greater mating success.

Wolf spider book lung physiology leads to an increased reliance on anaerobic metabolic pathways and, in spiders; the major anaerobic by-product is D-lactate (Prestwich 1983). During bursts of activity resulting from prey capture, predator evasion, or courtship, both local and systemic oxygen deprivation occurs resulting in an increased reliance upon anaerobic pathways (Ellington 1983; Weinstein & Full 1992; Schmitz & Harrison 2004). The activity patterns of wolf spiders are particularly interesting because these animals have lower rates of metabolism than other ectotherms of equivalent size (Anderson & Prestwich 1982; Anderson 1992; Gillooly et al. 2001). This is attributed to their poorly developed aerobic and highly developed anaerobic abilities (Linzen & Gallowitz 1975; Prestwich & Ing 1982; Prestwich 1983). The necessity of anaerobic metabolism is due to insufficient oxygen supply to mitochondria and the electron transport chain resulting in inadequate regeneration of $NAD^+$. During anaerobic conditions, sugars (e.g. glucose) are metabolized into 3-carbon molecules of pyruvate. Pyruvate is then reduced by NADH into lactate (and $NAD^+$). The production of $NAD^+$ is required for glycolysis (and energy production) to continue (Prestwich & Ing 1982; Prestwich 1983a). Aside from temperature, the production of lactate in the tissues has been suggested to effect optimal performance and ultimately activity patterns of terrestrial ectotherms (Bennett 1978; Prestwich 1988). Prestwich (1988) investigated the interrelationship between lactate accumulation and fatigue in two spider species (*Filistata hibernalis, Lycosa lenta*) during and after maximal activity. He describes wolf spider exhaustion in two phases; in the first 10s of maximal activity spiders slowed by over 50%, and then over the next 60-90s reach maximal exhaustion (2 min.), albeit at a slower rate. Preswich (1988) suggests that the initial phase of fatigue is due to the primary depletion of phosphagen stores (ATP) during the first 10 to 15 seconds of activity, and then followed by the accumulation of lactate which ultimately results in fatigue (collapse)

(Prestwich 1988). The decreased rate of activity and decreased rate of lactate production after 10-15s has been suggested to be due to the reliance on *de novo* synthesis of high energy phosphate (ATP) (Prestwich 1988). The accumulation of D-lactate was found to be constant or increasing after 10 seconds of activity, and the fastest running speeds were not associated with the highest rates of lactate accumulation it was also found that the duration of complete lactate removal ranged from 15 to 30 min during the recovery period (Prestwich 1988). Thus, the concomitant accumulation of lactate and wolf spider exhaustion after 2 minutes of maximal activity suggests lactate as the primary cause of wolf spider fatigue. This excessive utilization of anaerobic pathways during maximal activity has been shown to induce a strong metabolic acidosis, resulting primarily from lactate accumulation in the hemolymph of spiders (Prestwich 1988).

Biochemical reactions, typically those that are not catalyzed by enzymes, are under strong influence by the environment in which the biochemical reaction is taking place (Angilletta 2009). This is primarily due to the capability of a substrate achieving the free energy necessary to complete the reaction. Generally, as temperature increases, there is more energy available in the system which provides a higher ratio of substrate the capability to exceed the energy of activation ($E_a$) and thus, produce more product (Stipanuk 2006; Angilletta 2009). Enzymes reduce the ($E_a$) necessary for a specific reaction which decreases the dependence of biochemical reactions upon free energy from the surrounding environment (Stipanuk 2006). However, because enzymes function most effectively at normo-physiologic conditions, changes in pH and temperature significantly affect enzyme activity profiles (Hochachka & Somero 2002; Stipanuk

2006). Because spiders are ectotherms, they are subject to varying environmental temperatures as they explore their environment. Thus, temperature fluctuations likely have a significant effect on enzymatic function, altering biochemical reactions, and ultimately wolf spider performance. In addition, metabolic acidosis, or decrease in pH, induced by lactate disrupts enzyme function and $O_2$ equilibrium curves, potentiating the reliance upon anaerobic metabolism (Paul et al. 1994; Prestwich 1988b). These fluctuations in temperature and changing pH levels, induced by lactate, likely affect the efficiency of enzymes and ultimately affecting the ability of wolf spiders to perform maximally. During bursts of muscle contractions, such as during prey capture or predator evasion, oxygen levels within contractile muscle fall rapidly resulting in localized anoxia and concomitantly significant decreases in pH within the locomotory muscles (Ellington 1983; Schmitz & Harrison 2004). The evolution of spiders to rely on anaerobic metabolism as a "low cost" alternative to aerobic pathways has been suggested by their physiology and "sit-and-wait" style of prey capture (Levi 1967; Prestwich 1977; Prestwich 1983a; Paul et al. 1989). Localized anaerobiosis, coupled with insufficient hemolymph circulation, results in rapid fatigue (Ellington 1983; Paul et al. 1989; Paul et al. 1994). Therefore the ability to maximally sustain anaerobic metabolism as long as possible is crucial since the return to aerobic conditions is often unpredictable (Linzen & Gallowitz 1975; Bennett 1978; Prestwich & Ing 1982, Prestwich 1983a, b; Weinstein & Full 1992; Prestwich 2006).

Previous research on a closely related wolf spider species, *Schizocosa rovneri* (Davis 1989), and the model organism of this study, *S. ocreata* (Paniccia & Roberts unpubl), indicate that temperature significantly affects courtship. Preliminary analysis of baseline lactate

concentrations in spiders at rest and maximal lactate concentrations at exhaustion, suggest a differential accumulation by activity level (Ackers, O'hara, & Roberts unpubl). This disproportional accumulation of lactate by activity level may be influenced by temperature and this disproportionate accumulation of lactate, along with substrate temperature, may be affecting or influencing crucial courtship behaviors in *S. ocreata*. This thesis investigates the role of temperature on total lactate production in the wolf spider *S. ocreata*. The metabolic acidosis induced by lactate disrupts enzyme function and $O_2$ equilibrium curves. This further potentiates the reliance upon anaerobic metabolism in wolf spiders and is likely acting as a constraint on their ability to maximally reproduce, catch prey and avoid predators (Prestwich 1988b; Paul et al. 1994). Due to their reliance on anaerobic metabolism the overall goal of this research is to determine if a by-product of anaerobic metabolism, D-lactate, may be an additional constraint on wolf spider activity patterns. This hypothesis was addressed through two objectives; first, determine minimum and maximum lactate accumulations at rest and exhaustion, and second, determine the influence of temperature on lactate production. The relationship between lactate production and temperature may be non-linear, with lactate playing an increasingly important role in behavioral limitation at higher temperature. The second objective elucidates the possibility of disproportionate accumulation of anaerobic by-products across predetermined temperature ranges in this spider model.

**Objective 1: Determine minimum and maximum D-lactate production.** The objective of the first experiment was to establish D-lactate production levels (minimum and maximum) at room temperature. This provides a baseline for comparison to lactate production under thermal stress.

**<u>Objective 2:</u> Determine the degree to which lactate production is dependent on temperature through analysis of lactate concentration.** Building on the results of objective 1, this experiment focused on how the production of D-lactate varies with substrate temperature in *Schizocosa ocreata*. The production of D-lactate in the tissues has been suggested to effect optimal performance and therefore activity patterns of wolf spiders (Prestwich 1988; Hochachka & Lutz 2001). Most animals start and stop, or move intermittently between periods of high intensity activity and pauses. Lactate concentration has been cited to be significantly lower in animals that move intermittently rather than continually (Weinstein & Full 1992). Thus, intermittent recovery periods can alter metabolic capacities and endurance, and it stands to reason that spiders able to recover more efficiently at a given temperature will be able to tolerate performing at a level closer to the species maximum resulting in increased mating success.

**METHODOLOGY:**

**Spider Collection and Husbandry:** In April/May 2012, subadult *Schizocosa ocreata* were collected from The Dawes Arboretum in Licking County, Ohio at least 2 weeks prior to experimentation. All spiders were collected as juveniles in the field and raised to adulthood (only adults were used in experiments) at constant humidity, 25°C, and 12/12hr light:dark photoperiod. Spiders were housed individually and visually isolated from other spiders. Subjects were supplied with water *ad libitum*, fed 2-3 cricket nymphs twice per week with the last feeding 2-3 days prior to experimentation to ensure consistent metabolic status (Prestwich 1983). During temperature controlled experiments, spiders were allowed to acclimate,

undisturbed, at treatment temperature for at least 15 minutes prior to the start of a trial (Ackers, Zajd, & Roberts unpubl).

**Reagents:** All reagents were purchased from Fisher Scientific. Glycine buffer (500mM Glycine, 400mM Hydrazine Hydrate, pH 9.0), and nicotinamide-adenine dinucleotide (NAD) solution (Ca. 41mM β-NAD) were prepared weekly and stored stoppered in a refrigerator at 4°C. Perchloric acid (5.8N) and Potassium Hydroxide (1N) solutions were stored and are stable indefinitely at 4°C. D-(-)-Lactate dehydrogenase (D-LDH) solution (5mg protein/mL) was prepared freshly every 6 months and stored at 4°C in a sterile microcentrifuge tube.

**Substrate Temperature Modification:** A heating/cooling table modified to have up to four independently controllable zones was used for substrate temperature modification/regulation. This heating/cooling table was able to successfully maintain the substrate temperature at the predetermined (15, 25, 35°C) intervals.

**Objective 1: Obtaining and Analyzing D-lactate**

Each spider was placed in a 25mL Erlenmeyer flask and held at 25°C. Following the procedure of Prestwich (1982), the rest group was timed for 25 minutes during which the spiders stand still (or make very few and very small movements). After 25 minutes the spiders were immediately frozen with liquid nitrogen, removed from the flask, and placed in a 2ml micro-centrifuge tube. In the exhausted group, each spider was constantly stimulated to exhaustion for 2 minutes by prodding and then, quickly frozen. After freezing, spiders were placed in individual microcentrifuge tubes and stored in a -70°C freezer until further analysis. Prior to analysis, each spider was weighed, homogenized in 4°C 0.6 M $HClO_4$ for two minutes. This homogenate was separated via centrifugation at 3,500 rpm for at least four minutes. The

supernatant was neutralized using 4°C 6 M KOH, mixed, allowed to stand overnight at 4°C and then filtered through a glass fiber filter (Whatman 934 AH). Following the procedure outlined by Gawehn & Bergmeyer (1974), reaction vessels with glycine buffer, and $NAD^+$ solution were prepared prior to filtration to ensure rapid addition to the test tube. Once the filtrate (sample) was added to the reaction vessel, an absorbance value was read ($E_i$) using a BMG Labtech 96-well plate spectrophotometer (FLUOstar Omega). The buffer was treated with D-LDH initiating the reaction, which was allowed to run to completion (approximately 6hrs). A final absorbance ($E_f$) was read and used to calculate $\Delta E$ ($E_f - E_i = \Delta E$) and finally D-lactate concentration (µmole/ml). All reaction tubes were compared against a blank control which was prepared identical to the reaction tube however with doubly distilled water in place of sample.

**Objective 2: D-lactate and temperature**

Spiders were treated following the procedures previously outlined. In a two-way ANOVA design, spiders were arbitrarily subdivided into three temperature groups (Table 1) designed to be similar to thermal extremes experienced in the field, and two treatments (Exhausted or Rested). Analysis of D-lactate concentration was read and compared against an extinction curve. Calculations were preformed according to Gawehn & Bergmeyer (1974). Specific to experiment 2, spiders were contained within a ring apparatus on the heating/chill table.

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Temp. (°C) | 15°C | 25°C | 35°C |
| Rest (25min) | 5 ♂ | 5 ♂ | 5 ♂ |
| Exhaustion (2min) | 5 ♂ | 5 ♂ | 5 ♂ |

**Table 1: Experimental design. Treatments include 10 Spiders per group.**

**RESULTS:**

**Objective 1: Determination of minimum and maximum D-lactate production**

Using the procedure outlined by Gawehn & Bergmeyer (1974), It was found that the accumulation of D-lactate did not vary significantly according to whole spider mass (Figure 1). That is, larger spiders do not inherently have a higher concentration of lactate at either, rest or exhaustion. Although the accumulation of lactate was not correlated with mass, there is a significant difference in lactate production between rested (non-exhausted) and exhausted spiders at room temperature (~25°C) (Table 4; Figure 2, 25°C group). This is also to be expected and is supported by previous studies investigating lactate and maximal exercise (Prestwich 1983 a, b; Prestwich 1988 a, b; Schmitz 2004; Schmitz & Harrison 2004; Schmitz 2005; Prestwich 2006).

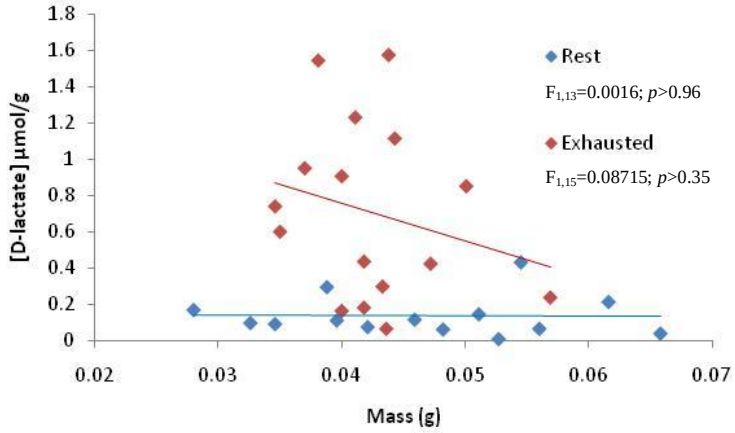


**Figure 1: Concentration of D-lactate by mass in rested and exhausted spiders.**

**Objective 2: Determine the degree to which lactate production is dependent on temperature through analysis of lactate concentration**

There was not a significant difference in the accumulation of D-lactate in *S. ocreata* across the temperature ranges investigated. Thus, our data do not suggest any temperature effects on maximal or baseline lactate concentrations (Figure 2) failing to reject the null hypothesis.

**DISCUSSION:**

There is a great deal of literature exploring the aerobic and anaerobic capabilities of ectothermic vertebrates (Bennett 1978; Hochachka & Lutz 2001; Bickler et al. 2002; Savina et al. 2009). However, far fewer studies are available that involve ectothermic invertebrates (Ellington 1983; Weinstein & Full 1992; Schilman et al. 2011), especially arachnids (Prestwich 1983a, b; Prestwich 1988a, b; Schmitz & Harrison 2004; Prestwich 2006). Wolf spiders (Lycosidae), like all spiders, are ectothermic, meaning that their body temperature corresponds to the temperature of the surrounding environment (Foelix 2010). Thus, their ability to perform both aerobically and anaerobically is largely dependent on external temperature. Wolf spiders perform a number of behaviors, many of which benefit males through sexual selection, but may result in higher physiological costs as well as increased direct costs from predation, parasitism, and competition (Zahavi 1975; Hamilton & Zuk 1982; Endler 1991; Magnhagen 1991; Andersson 1994; Møller & De Lope 1994; Ramos 2004; Hebets and Papaj 2005). The reliance on anaerobic metabolism in spiders as a "low cost" alternative to aerobic pathways has been suggested by their physiology (Prestwich 2006). Therefore the ability to

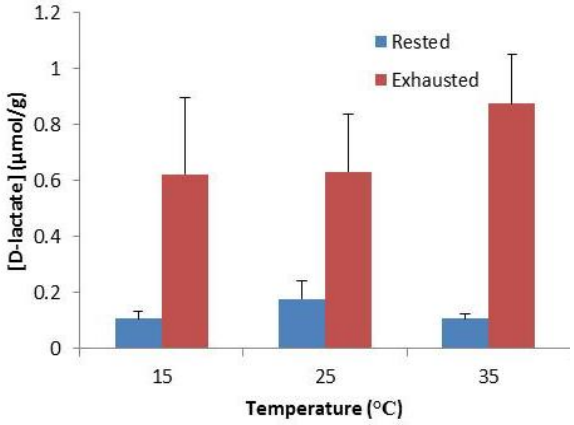


**Figure 2: Mean lactate concentrations (±SE) for exhausted and rested spiders across temperature treatments.**

| Source | DF | F Ratio | *p* |
|---|---|---|---|
| Temperature | 2 | 0.148 | 0.8634 |
| Treatment | 1 | 14.315 | 0.0008* |
| Temperature*Treatment | 2 | 0.777 | 0.4702 |

**Table 4: Two-way ANOVA results for lactate production by temperature (15, 25, 35°C) and treatment (rested, exhausted).**

maximally sustain anaerobic metabolism as long as possible is crucial since the return to aerobic conditions is often unpredictable (Bennett 1978).

During anaerobic metabolism, there are many proposed routes of energy production. Previous research on wolf spiders suggest that lactate dehydrogenase (LDH) and glycerol-3-phosphate dehydrogenase (GPDH) are likely the major contributors to energy production during anaerobic metabolism because they occur simultaneously in the cytosol resulting in competition for NADH, which is used in aerobic respiration or oxidative phosphorylation (Guppy & Hochachka 1978). This competition for a common energy source is regulated through inhibition and activation of particular enzymes. For example, during anoxic conditions when concentration of LDH is high, the flow of carbon and energy to oxidative phosphorylation is reduced. Thus, allowing for anaerobic metabolism to predominate as the primary energy source (Guppy & Hochachka 1978). It has been shown by Prestwich (1983a) that D-lactate is the major by-product of anaerobic metabolism in spiders accounting for over 97% of the total high energy phosphate production. Thus, previous research has clearly established utilization of the D-lactate pathway as the primary means of high energy phosphate production during anaerobic conditions in spiders. Spiders are unique, in that, they have very poor aerobic capacities and relatively well developed anaerobic capacities and, because of this, are incapable of long durations of intense activity: with maximal exhaustion reached in less than 2 min (Anderson & Prestwich 1982; Prestwich 1983 a, b; Bromhall 1987). This low capacity for high intensity activity has been suggested to be a constraint on wolf spider activity, and there have been two major suggestions as to the causes of this constraint: the fluid insufficiency hypothesis, and the accumulation of anaerobic by-products (Linzen & Gallowitz 1975; Prestwich 1983 a, b; Prestwich 1988 a, b). Of these two hypotheses, the accumulation of anaerobic by-products, such as D-lactate, has been

shown by Prestwich (1988 a, b) to be the major contributor to long term fatigue in wolf spiders, which has been investigated in this honors thesis.

The accumulation of lactate by temperature was investigated and minimum and maximum lactate concentrations at rest and at exhaustion were established. The data clearly demonstrates that there is a difference in lactate concentrations in exhausted and non-exhausted spiders. This is as expected, and consistent with existing literature on lactate and exercise (Prestwich 1983 a, b; Prestwich 1988; Weinstein & Full 1992). Previous research on ectotherms found a significant negative correlation between temperature and resting metabolic rate (Clarke & Fraser 2004; Gilooly et al. 2001). However, our data do not suggest that there are temperature effects on either maximal or baseline lactate concentrations (Figure 2). It is possible that maximal lactate tolerance may be independent of environmental temperature. Although absolute lactate concentration is not significantly affected by substrate temperature and maximal lactate accumulation may be independent of environmental temperature; the likelihood that spider fatigue is caused by buildup of various metabolites other than D-lactate (or other mechanisms not yet known) is not likely based on work by Prestwich (1983, 1988). Larger spiders do not inherently have higher concentrations of lactate because maximal lactate accumulation is not affected by total spider mass (Figure 1), and it is likely that there is a maximal level of lactate that male *S. ocreata* locomotory muscle can produce. Because this maximal level of lactate concentration is consistent between males, it is not likely affecting male wolf spider behavior. While absolute lactate concentration is not significantly affected by substrate temperature, latency to maximum lactate production could be. The latency to maximum lactate production, or the duration of maintaining maximal activity before fatigue may be increasingly important with varying temperatures (Ellington 1983; Weinstein & Full 1992; Clark & Fraser 2004). Thus, it is

possible that a temperature dependence upon latency to fatigue is potentially affecting male wolf spider behavior and males may seek optimal temperature sites that allow extended courtship, thus enhancing chances of attracting a mate. This line of reasoning is supported by Weinstein and Full (1992), who investigated the effect of start-stop, or intermittent exercise in the ghost crab *Ocypode quadrata*. When forced to exercise continuously, crabs fatigued quickly and covered a distance of only 135 meters. When crabs were subject intermittent exercise (2-min exercise/ 2-min pause) total time to fatigue and total distance traveled increased significantly (Weinstein & Full 1992). Additionally, significant amounts of lactate were removed during the pause periods (Weinstein & Full 1992). This suggests that during the pause period, lactate accumulated during exercise can be cleared and energy sources can be renewed. Thus, behaving intermittently can significantly change the endurance limitations and permit behaviors of high intensity in the ghost crab *Ocypode quadrata*. There is a great deal of evidence in support of the idea that increased rate, duration, and complexity of male courtship signals correspondingly increases male mating success (reviewed by Andersson 1994; Alcock 2005). With rate of male courtship displays playing a crucial role in mate choice (Jackson 1977, 1982; Clark & Uetz 1992; McClintock & Uetz 1996; Scheffer *et al*. 1996) males able to minimize time of recovery after exhaustion, or maximize anaerobic endurance prior to exhaustion, will likely increase mating success. Thus, males may seek optimal temperature sites that allow extended courtship thus increasing mating opportunities.

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